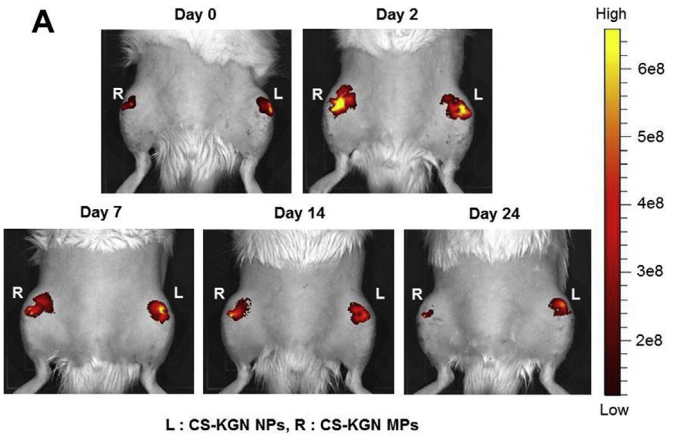

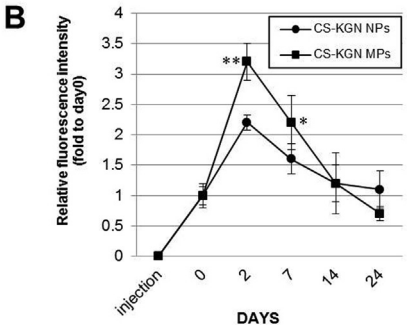

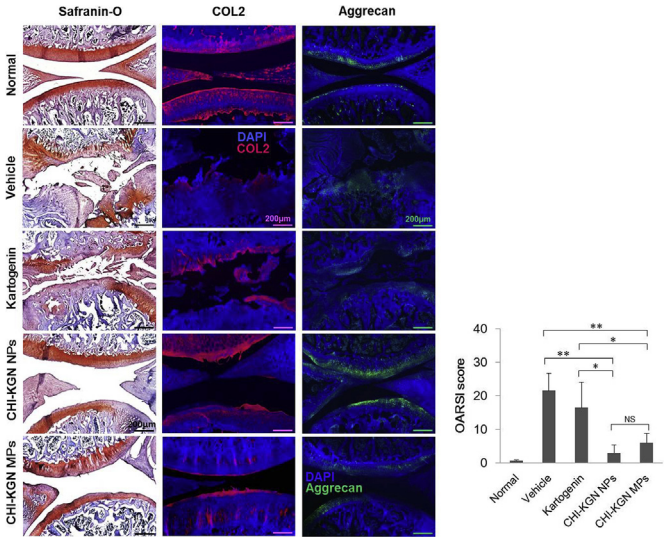

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VIRAL EXPRESSION OF TSG-6 CAN STIMULATE OSTEOPHYTE FORMATION IN EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Tumor necrosis factor-inducible gene 6 (TSG-6, also named TNFAPI6) is upregulated during experimental arthritis in mice (Geurts et al. 2009). The therapeutic efficacy of TSG-6 has been shown in multiple animal models for rheumatoid arthritis, reducing both inflammation and cartilage damage. Recent studies suggest that inflammation can also play a role in early osteoarthritis (OA) pathogenesis (Ayral et al. 2005). Therefore, TSG-6 therapy might also be an effective treatment in inflammatory OA. In this study, we analyzed the expression of TSG-6 during experimental arthritis and explored the effects of TSG-6 gene therapy in a murine model of inflammatory osteoarthritis.
Methods: TSG-6 gene expression was determined in microarrays of collagenase-induced osteoarthritis at day 7 and day 14 after induction. The murine TSG-6 gene was cloned in a lentiviral and adenoviral vector. Freshly isolated bone marrow-derived dendritic cells (BMDCs) were transduced with the lentiviral vector and subsequently differentiated into osteoclasts on bone slices with M-CSF and RANKL. After 10 days, bone slices were washed and stained using Coomassie Blue and bone resorption was determined using the Leica Application Suite software. To study the effects of TSG-6 in experimental knee osteoarthritis, mice received two intra-articular injections of collagenase. Four days prior to and 20 days after arthritis induction, mice were injected intra-articularly with TSG-6 adenovirus. At day 7, inflammation was assessed using fluorescent Prosense probes. At day 42, knee joints were analyzed by X-ray and histological assessment.
Results: TSG-6 was upregulated in collagenase-induced OA (2,6x at day 7 ($P<0.05$) and 2.5x at day 14 ($P=0.55$)). BMDCs transduced with TSG-6 lentivirus showed strong expression of TSG-6. Bone resorption by BMDC-derived osteoclasts was significantly reduced (20.1% surface erosion with control virus to 10.4%, $p=0.01$), providing a possible mechanism for the therapeutic effects in rheumatoid arthritis models. At day 7 of collagenase-induced osteoarthritis, no difference in inflammation was detected using the Prosense probes. At day 42, no improvement on cartilage damage was seen, but X-ray analysis showed strong osteophyte formation at the femur/tibia region in the knee joint. Histological analysis showed that the osteophytes contained both bone and cartilage.
Conclusions: Viral expression of TSG-6 can reduce the bone resorption activity by BMDC-derived osteoclasts. The expression of TSG-6 by synovial cells during experimental osteoarthritis results in the formation of osteophytes. These results imply a causative role for TSG-6 in osteophyte formation, supporting the recent finding that TSG-6 activity is associated with radiographic progression of OA (Wisniewski et al. 2014).

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INTRA-ARTICULAR HYALURONIC ACID DELAYS TOTAL KNEE REPLACEMENT IN PATIENTS WITH KNEE OSTEOARTHRITIS: EVIDENCE FROM A LARGE U.S. HEALTH CLAIMS DATABASE

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Purpose: To evaluate the impact of one or more courses of treatment with intraarticular (IA) hyaluronate (HA) compared to no IA HA injections on the time to total knee replacement (TKR) on patients with osteoarthritis (OA), using a large administrative claims database.
Methods: Retrospective analysis used the IMS PharMetrics Plus claims database of approximately 79 million patients to identify patients with OA of the knee who received TKR within a 6 year selection window (2007-2013). The initial OA diagnosis in the database was the index date and included patients with knee OA who had continuous enrollment from OA diagnosis until TKR. Kaplan-Meier survival analysis was used to measure time-to-TKR. Subsets were defined by the number of courses of treatment received, ranging from 0 (no IA HA), 1, 2, 3, 4, to $\geq$5 HA courses. Median time was the time (in years) at which 50% of patients in a cohort had received TKR. Log-rank tests were used to compare different cohorts.
Results: The database included 182,022 patients with knee OA and TKR. Of these, 131,673 (72.3%) patients had no HA, and 50,349 (27.7%) received at least one course of IA HA. Age, sex, and Charlson comorbidity scores were similar among subsets. Of those receiving IA HA, a total of 36,861 patients (73.2%) received 1 HA course, 8893 (17.7%) received 2 courses, 2,783 (5.5%) received 3 courses, 1052 (2.1%) received

4 courses, and 760 (1.5%) received 5 courses or more. The time from diagnosis to when 50% of the subset received TKR was significantly longer (p1 year, an average of 9 months longer than those with no IA HA. Patients who received ≥5 courses had a delay in TKR by 3.6 years.
Conclusions: Among 182,022 patients with knee OA, those who received IA HA had a significantly longer time before TKR. More courses of IA HA injections were associated with a longer time to TKR. This study suggests a significant clinical benefit from use of IA HA for OA as delay in time to TKR can have important clinical and economic implications.

Median Time from knee OA diagnosis to TKR by number of courses of IA HA

	No IA HA	1	2	3	4	5+
Days to TKR	114	386	648	875	1054	1312
Years to TKR	0.3	1.1	1.8	2.4	2.9	3.6

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EVALUATION OF THE CLINICAL EFFICACY OF AUTOLOGOUS CONDITIONED SERUM IN PATIENTS WITH COXARTHROSIS

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Purpose: The aim of this open study was a comparative assessment of the effectiveness of local therapy with autologous conditioned serum (ACS) and low molecular weight hyaluronic acid (LHA) in patients with coxarthrosis.
Methods: The study included 60 patients with firm coxarthrosis in accordance with ACR criteria at the age of 55,5 ± 8,7. The main group (ACS treatment) consisted of 33 (55%) persons. Experimental group (LHA treatment) consisted of 27 patients who are comparable with the main group patients in terms of age, BMI, radiographic stage, disease duration and severity of clinical indicators. ACS was prepared in accordance with established method and injected intraarticularly (2.5 ml twice a week for three weeks). LHA treatment consisted of 3 weekly intraarticular injections of 40 mg sodium hyaluronate each. All intra-articular actions were performed with ultrasound control. Treatment efficiency was evaluated after 1,3 and 6 months after treatment, the following criteria were used: bodily pain dynamics in accordance with VAS, morning stiffness module and Womac index functional scale, and Lequesne index. “Area under the curve” (AUC) approach with estimation of treatment efficiency prolongation for 6 months (AUC6) was used for evaluation of clinical effect retention.
Results: A decrease in pain syndrome intensity according to VAS in hip joints at 1, 3 and 6 months of treatment in both compared groups was recorded.However, a significant regression was observed in the treatment of pain with ACS in comparison with LHA.After 1 month the decrease of pain severity in accordance with VAS was comparable (9.1%, $p = 0.40$), after 3 and 6 months, pain severity was higher in the LHA group compared with ACS group (+ 52.5%, $p = 0.009$ and + 33.1%, $p = 0.047$ respectively). AUC6 in case of ACS treatment was 35,6% ($p = 0,011$) higher compared with LHA treatment. Extension of the clinical effectiveness expressed via "morning stiffness" module of the Womac index AUC6 indicator was 55.9% ($p=0.003$) higher in case of ACS treatment compared with LHA treatment. Womac functional scale in case of ACS improved through all three control timepoints (-23.1% -28.5% -39.1%; $p = 0.001$). Same dynamics was observed in LHA group as well (-35.6%, -26.4%; $p = 0.001$ and -20.4%; $p = 0.005$), however after 6 months of monitoring more significant improvement of articular function was recorded within ACS group compared with LHA (18.3%, $p = 0.044$). The overall clinical efficacy in accordance with Lequesne index with a six-month monitoring was comparable in both groups, the difference came up to 8,8% ($p = 0,65$).
Conclusions: Intensity of pain, stiffness, functional status and overall clinical severity of coxarthrosis significantly and consistently decreased during therapy with ACS and LHA.However, favorable changes of local therapy with LHA were inferior in duration of conservation of treatment effect of ACS.

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REFINEMENT OF PRECLINICAL STUDIES FOR VISCOSUPPLEMENTATION THERAPY EVALUATION: INTEREST OF COMPLEMENTARY TECHNIQUES TO EVALUATE CARTILAGE IN A RABBIT MODEL OF EARLY OSTEOARTHRITIS

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Purpose: One of the major challenges of the viscosupplementation (VS) therapy is the development of more efficient formulations and, to this end, the use of animal models of osteoarthritis (OA) is still mandatory. The assessment of VS efficacy is challenging mainly since its structural effects are subtle. In order to refine preclinical VS efficacy studies and reduce the number of animals used, there is a crucial need for more sensitive and discriminant evaluation tools. In this study, we especially focused on complementary techniques to evaluate OA cartilage in a rabbit model of early OA.
Methods: Cranial cruciate ligament transection (ACLT) was performed in the left knee of white New-Zealand rabbits (n=12) to induce traumatic OA. One week post-ACLT and then weekly for 5 weeks, the operated knees of 6 rabbits were injected with a hyaluronic acid (HA) containing commercial formulation (Ostenil®, HA group). One group was injected with saline (operated-control group, n=6). The contralateral right knees (n=8) were used as unoperated-controls. End-point evaluation was done at the 6th week post-ACLT and included: gross and histological scoring of cartilage lesions, measurement of cartilage thickness by Equilibrium Partitioning Iodine Contrast micro-Computed Tomography (EPIC μ-CT) as well as the evaluation of the surface by Scanning Electron Microscopy (SEM).
Results: Gross and histological scorings showed statistical differences between operated and unoperated knees; however, no difference between the HA and operated-control groups was evidenced. SEM revealed that unoperated-control samples had normal smooth to rough surfaces with discreet cable-like structures. Cartilage from the operated knees presented rough surfaces and clearly visible cable-like structures, which might be the sign of cartilage matrix erosion. Finally, mean cartilage thickness and volume measured by EPIC μ-CT were comparable for the 3 groups. Interestingly, the use of the thickness distribution representation showed clear differences in the intact cartilage occurrences (i.e. thickness higher or equal to 0.9 mm). Indeed, intact cartilage proportion decreased statistically from 20% in the unoperated-control group to 10% in the operated-control group. In addition, intact cartilage proportion in the HA treated knees was equivalent to unoperated-control group, highlighting a moderate efficacy of HA which was detected neither by histology nor by SEM.
Conclusions: Complementary techniques were implemented to evaluate cartilage lesions in a rabbit model of early OA in order to refine preclinical VS efficacy studies. This study points out that classical evaluation tools (macroscopy and histology) are sensitive enough to discriminate between cartilage from unoperated and operated groups in early OA but that EPIC- μCT also permits the detection of the subtle structural effects of HA, validating this technique as a powerful evaluation tool.

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EVIDENCE OF *IN VIVO* DRUG DELIVERY VIA THE TAT PROTEIN TRANSDUCTION DOMAIN

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Purpose: Drug delivery to synovial joints is a major limitation for developing effective treatments for osteoarthritis (OA). Synovial joint anatomy includes large pores that allow direct transport between synovial fluid and fenestrated capillaries. This transport preferentially selects larger molecules to remain within the joint and exports smaller